HK3

PDB:3HM8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC028129
Entry Clone Source:MGC
SGC Clone Accession:HPC041-C10
Tag:N-terminal tag: mgsshhhhhhssglvpr*gs
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:HK3:R480-R922
Tag was removed.
Sequence:

```
gsRRLLEETLAPFRLNHDQLAAVQAQMRKAMAKGLRGEASSLRMLPTFVRATPDGSERGDFLALDLGGTNFRVLLVRVTTGVQITSE
IYSIPETVAQGSGQQLFDHIVDCIVDFQQKQGLSGQSLPLGFTFSFPCRQLGLDQGILLNWTKGFKASDCEGQDVVSLLREAITRRQ
AVELNVVAIVNDTVGTMMSCGYEDPRCEIGLIVGTGTNACYMEELRNVAGVPGDSGRMCINMEWGAFGDDGSLAMLSTRFDASVDQA
SINPGKQRFEKMISGMYLGEIVRHILLHLTSLGVLFRGQQIQRLQTRDIFKTKFLSEIESDSLALRQVRAILEDLGLPLTSDDALMV
LEVCQAVSQRAAQLCGAGVAAVVEKIRENRGLEELAVSVGVDGTLYKLHPRFSSLVAATVRELAPRCVVTFLQSEDGSGKGAALVTA
VACRLAQLTR
```

Vector:pET28a-LIC (GI:145307000)

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Ni-NTA beads and incubated at 4°reeC on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were

then washed with washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-trough was collected and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions were collected and digested with TEV protease. TEV protease was removed from the treated protein sample by adding 100 µL 50% flurry of Ni-NTA beads and the sample was purified with supderdex-75 gel filtration again. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 99%.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL Binding buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:33.50 mg/mL
Ligand
5mM Glucose-6-phosphate, 5mM ADP, 10mM Mg2+**MassSpec:**Expected mass for native protein: 48318.6 (tag cut), measured 48323.52.

Uncut expected: 50069.46, measured: 50024.64, 50082.45
Crystallization: Crystal used for data collection was grown in 16% PEG 3350, 0.20 M Ammonium Citrate, 0.1 M HEPES pH 7.2, using hanging drop vaporization method. Protein stock solution was added with 5 mM Glucose-6-phosphate, 5mM ADP/Mg, 10 mM MgCl2, 20mM TCEP, and supplemented with 1:100 (w/w) trypsin, then 0.5 µL protein solution and 0.5 µL well solution was mixed and sealed in a Linbro 24-well plate containing 500 µL well solution in each well.
NMR Spectroscopy:
Data Collection:
Data Processing: